and last position of the CDR. The population will also contain sets corresponding to positions 2 and 3, 2 and 4, 2 and 5, through the set corresponding to the second an last position of the CDR. Likewise, the population will contain sets of double position changes corresponding to all pairs of position changes beginning with position three of the CDR. Similar pairs of position changes are made with the remaining sets CDR amino acid positions. Therefore, the population will contain species that represent all pairwise combinations of amino acid position changes. In a simialar fashion, populations corresponding to sets of changes representing all triple and quadruplet changes along a CDR can similarly be targeted for grafting into the variable region frameworks using the methods of the invention.

The above populations of CDR variant species can be targeted for any or all of the CDRs which constitute the binding pocket of a variable region. Therefore, an acceptor variable region framework targeted for relevant amino acid positions changes as described previously, can be targeted for the simultaneous incorporation of donor CDR variant populations at one, two or all three recipient CDR locations. The choice of which CDR or the number of CDRs to target with amino acid position changes will depend on, for example, if a full CDR grafting into an acceptor is desired or whether the method is being performed for optimization of binding affinity. Many grafting procedures will generally employ the grafting of all three CDRs, where at least one of the CDRs will contain amino acid positions changes. Generally however, all of the donor CDRs will be populations containing amino acid position changes. Converesly, and as described further below, optimization procedures can employ CDR variant populations

corresponding to any or all of the CDRs within a variable region.

Another approach for selecting donor CDR amino acids to change for conferring donor CDR binding affinity onto an antibody acceptor variable region framework is to select known or readily identifiable CDR positions that are highly variable. For example, the variable region CDR 3 is generally highly variable due to genetic recombination. This region therefore can be selectively targeted for amino acid position changes during grafting procedures to ensure binding affinity reacquisition or augmentation when made together with relevant acceptor variable framework changes as described previously.

In contrast, CDR residues that appear conserved or have been empirically determined to be non-mutable by functional criteria will generally be avoided when selecting residues in the CDR to target for change. It should be noted however, that apparent non-mutable residues can nevertheless be successfully changed using the methods of the invention because the populations of altered variable regions contain from a few to many amino acid position changes in both the framework regions and in the CDR regions. As such, the CDR grafted variable regions identified by binding affinity are a result of the all the changes and therefore, all the interactions of residues introduced into a particular species. Therefore, suboptimal residues incorporated at, for example, an apparent non-mutable position can be counteracted and even augmented by amino acid substitutions elsewhere in the framework regions or in other CDRs.

Similarly, because the methods of the invention for CDR grafting, affinity reacquisition and affinity optimization employ the production and screening of diverse populations of variable region species generated from an acceptor framework and donor CDR variants, there are numerous effects on binding affinity that will occur due to the combined interactions of two or more amino acid changes within a single variable region species. For example, the affect of amino acid changes in either a framework region or CDR that are inherently beneficial can be masked or neutralized due to surrounding authentic parent residues or due to their context in a heterologous region of a grafted antibody. However, second site changes in the surrounding residues or the heterologous regions can unveil the beneficial characteristics of the latent residue or residues. Such second site changes can occur, for example, in both proximal and distal heterologous or homologous region sequences.

For example, if the beneficial residue is in a grafted CDR region, the proximal heterologous sequences would be the adjacent framework regions whereas distal heterologous regions would be framework regions separated by an adjacent CDR. In this specific example, a proximal homologous region would be the surrounding residues within the grafted CDR harboring the beneficial change whereas the remaining CDRs are examples of distal homologous regions. By analogy, the opposite would be true for a inherently beneficial residue in a framework region. Specifically, proximal homologous region sequences would be located in the same framework region and distal homologous sequences would be in any of the other framework regions. Proximal heterologous region sequences would be in the adjacent CDR or CDRs whereas nonadjacent CDRs constitute distal heterologous region